nonblocking antibody BV12 recognizes both. Thus, dimerization and homophilic binding might contribute to both the junctional localization and adhesive function of JAM-1.

Claudins

Extremely complex TJs are found between cerebral endothelial cells that form the blood–brain barrier (BBB)[8]. Britta Engelhardt (Bad Nauheim, Germany) explained that during inflammation, such as in experimental autoimmune encephalomyelitis (EAE), the specialized properties of the BBB are frequently lost, resulting in brain edema. In EAE, the specific loss of immunostaining for the TJ molecule claudin-1 from inflamed cerebral vessels was observed, whereas the localization of the endothelial-specific claudin-5 and other TJ molecules, such as occludin and ZO-1, was conserved. In an *in vitro* model of the BBB, this selective loss of claudin-1 correlates directly with the opening of endothelial TJs (Fig. 1). These data demonstrate that claudin-1 plays a central role in determining the permeability of TJs of the BBB.

Conclusions

It is an old, but ever more valuable, concept that interfering with the functions of vascular cells and/or leukocytes can stop the progression of chronic inflammatory diseases. Although existing drugs block some aspects of the inflammatory mechanisms, they lack specificity and produce side-effects. Thus, new therapeutic targets in vascular biology are necessary to allow the development of better and more-specific therapies. The above examples suggest several new mechanisms involved in inflammatory diseases. Understanding the molecules and signal-transduction pathways that regulate vascular cell–cell junctions, vascular permeability and the transmigration of leukocytes will point to novel therapeutic targets for the specific inhibition of inflammatory diseases.

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The cited participants of the meeting contributed actively to this report. We regret that, owing to space constraints, the text represents only a small selection of the presentations.

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IL-10 and its homologs: important immune mediators and emerging immunotherapeutic agents

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The 3rd International Workshop on IL-10 and Related Molecules was held at Charité Berlin, Germany from 4–7 April 2001.

In 1989, Mosmann and colleagues first described a cytokine that is produced by T helper 2 (Th2)-cell clones and inhibits the synthesis of interferon γ (IFN-γ) by Th1-cell clones[1]. Today, this 'cytokine synthesis inhibiting factor' (CSIF) is known as interleukin-10 (IL-10), and a capacity to produce IL-10 has been demonstrated for various cell populations, including certain T-cell subsets [e.g. Th2 and T cytotoxic 2 (Tc2) cells], B cells, monocytes, macrophages and keratinocytes. IL-10 was considered to be a purely deactivating, immunosuppressive and anti-inflammatory cytokine initially, but recent investigations have demonstrated more-complex characteristics. In addition, novel homologs of IL-10, derived from mammalian as well as viral genomes, have been described.

The role of IL-10 in disease and its use as a therapeutic agent

Owing to its anti-inflammatory and immunosuppressive properties, IL-10 became a candidate for the therapy of several immunological diseases characterized by a Th1-type cytokine pattern, such as inflammatory bowel disease, rheumatoid arthritis, psoriasis and transplant rejection. Overall, early Phase II clinical trials using recombinant human IL-10 have demonstrated the safety and efficacy of this therapy for all of these indications. However, whereas a positive response to treatment with IL-10 was observed for psoriasis (K. Asadullah, Berlin and K. Reich, Goettingen, Germany), psoriatic arthritis did not improve considerably (I. McInnes, Glasgow, UK). Interestingly, it was demonstrated that long-term low-dose IL-10 therapy after the clearance of active psoriasis by conventional therapy is very effective at preventing a relapse and prolonging the disease-free interval (K. Asadullah). These data suggest that the immunoregulatory activity of IL-10 (e.g. the long-lasting expansion of the

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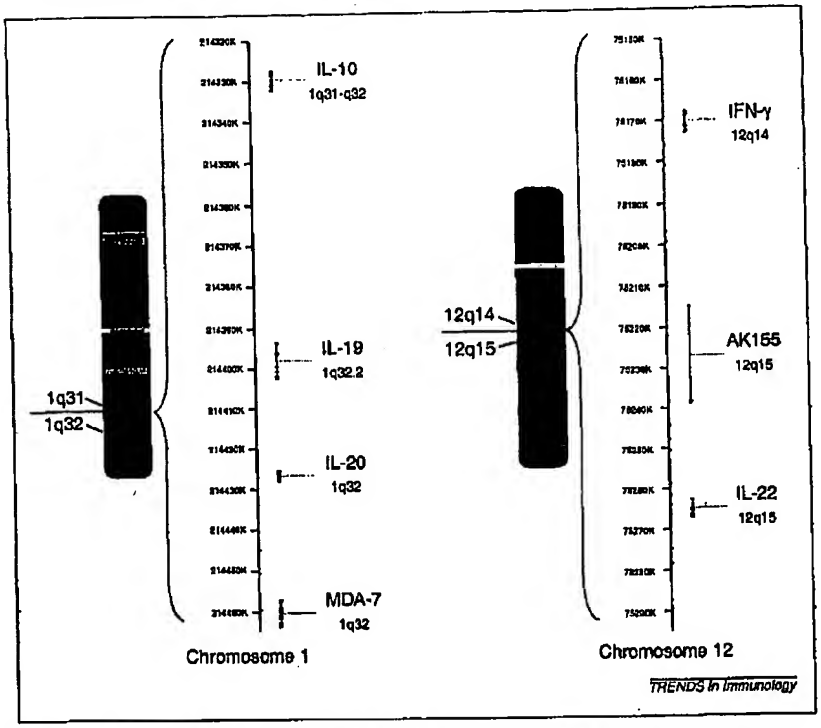

Fig. 1. The genomic localization of the genes encoding interleukin-10 (IL-10), interferon γ (IFN-γ) and the novel IL-10 homologs on human chromosomes 1 and 12. Abbreviations: AK155, Andrea Knappe 155 gene; MDA-7, melanoma differentiation-associated gene 7.

number of IL-4-producing T cells and decreased levels of CD80/CD86) might be more powerful than its anti-inflammatory properties [e.g. decreased synthesis of tumor necrosis factor (TNF) and/or IFN-γ]. It remains to be determined whether similar prophylactic approaches might be as effective and safe in the treatment of other immune diseases.

The delivery of IL-10 might be improved by gene therapy. Intragraft overexpression of IL-10 by adenoviral gene transfer prolongs graft survival in several models (T. Ritter, Berlin, Germany and S. Qian, Pittsburgh, PA, USA). However, this approach is limited by the high inflammatory potency of adenoviral vectors and the difficulties in generating the desired concentration of IL-10. A novel approach to target IL-10 to the site of undesirable inflammation is the use of polyclonally stimulated or antigen (Ag)-specific T-cell lines (C. van Montfrans, Amsterdam, the Netherlands and T. Ritter, respectively) that are retrovirally transduced with IL-10 and express specific tissue-homing receptors.

The role of IL-10 in allergic and atopic disorders seems to be complex. On the one hand, IL-10-mediated peripheral T-cell tolerance induced by allergen-specific immunotherapy, as well as by exposure to natural Ags, is considered to play a key role in the control of specific immune responses to high doses of Ag and/or allergen (C. Akdis, Davos, Switzerland). On the other hand, a lack of effectiveness of systemic IL-10 therapy was clearly demonstrated for atopic dermatitis (K. Reich). This might point to distinct underlying immunological mechanisms in the different types of allergies and atopic disorders.

It has been shown that IL-10 plays an important role in the pathogenesis of post-traumatic immune deficiency. Systemic inflammation is counter-regulated by the release of IL-10 and other immunosuppressive cytokines that are important to prevent septic shock. This counter-regulation is realized at the level of the macrophage but also the neuro–immune pathways, including the sympathetic nervous system, the vagal nerve and the hypothalamic–pituitary–adrenal axis (S. Rupprecht, Berlin, Germany). Excessive counter-regulation can lead to impairment of the immune system ('immunoparalysis') contributing to the high rate of late mortality seen in patients in intensive care.

Genetic polymorphisms strongly influence the capacity for the secretion of IL-10 by immune cells. Conflicting data have been published on 'high'- and 'low'-producer genotypes. This might be explained, at least in part, by the observation that specific polymorphisms in the promoter region of the gene encoding IL-10 have different influences on the expression of IL-10 depending on the particular trigger and type of cell involved (R. Kay, Dundee, UK). Several polymorphisms of the gene encoding IL-10 are associated with the incidence and severity of various immune diseases, including autoimmune diseases, transplant rejection, infections and lymphomas (J. Eskdale, Glasgow, UK; S. d'Alfonso, Milano, Italy; J. Cavot, Newcastle, UK; A. Vergopoulos, Berlin, Germany; and J. May, Tuebingen, Germany). The workshop developed a nomenclature for the described polymorphisms to facilitate the comparison of the data (G. Gallagher, Glasgow, UK).

IL-10 and regulatory T cells

The existence of regulatory T cells has been known for several years. However, the subgroup of T cells that these regulatory cells represent and the mechanisms of their inhibitory function remain to be determined. The production of IL-10 or transforming growth factor β (TGF-β) is thought to be important for the generation and/or action of regulatory T cells. The combination of cytokines expressed by regulatory T cells can depend on the manner by which they were generated and the type of dendritic cell (DC) used (M. Grazia-Roncarolo, Milano, Italy and A. Enk, Mainz, Germany). A. O'Garra (Palo Alto, CA, USA) described a completely different approach to the generation of regulatory T cells by treating Ag-presenting cells (APCs) with vitamin D_3 and dexamethasone. The inhibition of activation of nuclear factor κB (NF-κB) in APCs blocks their expression of pro-inflammatory genes and 'danger' signals, which leads to an increased generation of IL-10-producing

Table 1. The properties of IL-10-related molecules[a]

IL-10 homolog	Cellular source	Biological effects		Receptors
		In vivo	*In vitro*	
IL-19	Activated monocytes (LPS- or GM-CSF-stimulated)	ND	No effects on cytokine synthesis by PBMCs	ND
IL-20	LPS-stimulated PBMCs and monocytes (?)	Overexpression in mice causes retardation of growth and development, skin abnormalities and neonatal lethality	Enhancement of IL-1β-induced expression of inflammation-related genes (e.g. HaCaT)	IL-20Rα and IL-20Rβ
IL-22	Activated T cells (Th1 ?), IL-9-stimulated mast cells and mesangium cells	Application in adult mice induces an acute phase response and basophilia in proximal renal tubules; it acts as an autocrine factor for mesangium cells	Induction of an acute phase response (hepatoma cell lines), inhibition of IL-4 production by Th2 cells and activation of STAT3 in hepatocytes	IL-22Rα and IL-10Rβ
AK155	T-cell lines (CD4⁺ and CD8⁺), herpes virus saimiri-infected T cells and activated monocytes (?)	ND	ND	ND
MDA-7	Melanoma cells, skin fibroblasts and activated PBMCs (LPS- or PHA-stimulated)	Antitumor effects	Irreversible growth arrest of tumor (induction of apoptosis or differentiation), inhibition of angiogenesis and induction of expression of Th1- type cytokines in PBMCs (?)	ND

[a]Abbreviations: AK, Andrea Knappe; GM-CSF, granulocyte–macrophage colony-stimulating factor; IL, interleukin; LPS, lipopolysaccharide; MDA, melanoma differentiation associated gene; ND, not determined; PBMC, peripheral blood mononuclear cell; PHA, phytohemagglutinin; Th, T helper; STAT3, signal transducer and activator of transcription 3.

regulatory T cells in coculture. So far, it has been difficult to propagate these *in vitro*-generated regulatory T cells efficiently. The addition of IL-15 (instead of IL-2) seems to improve the clonal expansion of regulatory T cells (M. Grazia-Roncarolo).

IL-10 signaling and molecular mechanisms of inhibitory function

Although the immunosuppressive effects of IL-10 have been known for some time, there have been conflicting reports about the molecular basis of this inhibition. The nuclear translocation of the transcription factor NF-κB heterodimer is considered to be responsible for most of the transcriptional activity resulting from stimulation with lipopolysaccharide (LPS). The induction of the activity of mitogen-activated protein (MAP) kinases (e.g. p38) might play a role in the stabilization of mRNA or the ease of translation of cytokine mRNAs. Therefore, both NF-κB and MAP kinases are putative targets for the inhibitory action of IL-10; their possible inhibition has been studied by several groups with very different results.

These differences were reflected in talks given at the IL-10 workshop. IL-10 blocks the nuclear translocation of the p65 subunit of NF-κB, whereas the nuclear translocation of the p50 subunit remains intact, leading to the accumulation of inhibitory p50 homodimers in the nucleus (F. Driessler, Berlin, Germany). T. Mijatovic (Brussels, Belgium) demonstrated that in mouse macrophages, IL-10 mainly inhibits the expression of TNF at the post-transcriptional level. The 3′ untranslated region (UTR) of the TNF mRNA (containing AU-rich regions), as well as the 5′UTR, seem to be necessary for the complete inhibitory function of IL-10. That these different observations depend very much on the model used was discussed by B. Foxwell (London, UK). There are obvious differences between cell lines and primary cells. Both transcriptional and post-transcriptional inhibitory mechanisms are employed by IL-10 in human primary monocytes using adenoviral-delivered reporter constructs. The dominant mechanism depends on the time point at which IL-10 is added. If the cells are preincubated with IL-10, transcriptional inhibition dominates, but when IL-10 is added simultaneously with LPS, the 3′UTR is required for inhibition by IL-10, suggesting a post-transcriptional mechanism. Murine macrophages showed only the 3′UTR-mediated inhibition, regardless of the timing of

Key outcomes of the meeting

- IL-10 has been shown to be effective and safe in several clinical trials (e.g. for the treatment of rheumatoid arthritis) although it was less effective than therapy with anti-TNF mAb.
- In the treatment of psoriasis, IL-10 reversed acute disease effectively and prevented the relapse of disease activity. This might represent a novel approach for therapy with IL-10.
- It is possible to expand IL-10-driven regulatory T-cell populations *in vitro* using IL-15, without the loss of their suppressor function.
- IL-10 upregulates the expression of >300 genes, suggesting the complexity of its signaling.
- Several IL-10 homologs have been described. None of these appear to share the immunoregulatory and anti-inflammatory properties of IL-10. However, they have interesting biological activities including: antitumor effects, the induction of expression of acute-phase proteins and the stimulation of keratinocytes to proliferate.

exposure to IL-10, suggesting underlying species-specific differences in the mechanism of inhibition by IL-10.

The activation of signal transducer and activator of transcription 3 (STAT3), which is strongly influenced by signaling through the IL-10 type I receptor (IL-10RI), plays a key role in mediating the effects of IL-10 (M. Cassatella, Verona, Italy and R. de Waal Malevyt, Palo Alto, CA, USA). There is a region in the C-terminus of the IL-10R1 that (in addition to the activation of STAT3) is necessary for the immunosuppressive function of IL-10 in macrophages but dispensable for the activating function of IL-10 in B cells (B. Weaver, St Louis, MO, USA). This region is not involved in activating the Jak–STAT pathway. The Weaver group has identified an IL-10-responsive gene (TIGER), expression of which is dependent on the presence of this C-terminal region. This should help to identify the downstream signaling events.

The hunt is now on to find those genes that are mediators of the immunosuppressive function of IL-10. Some groups have used differential mRNA analysis to address this question. Preliminary results of some candidate genes were presented (L. Williams, London, UK), but there is a long way to go to prove the function of those genes whose expression is regulated by IL-10 and to understand the way in which they act at the molecular level.

IL-10-related molecules

During recent years, molecules with homology to IL-10 have been described, mostly as a result of *in silico* work. At the workshop, an update of this interesting group of molecules encoded by mammalian or viral genomes was given by several groups (R. de Waal Malevyt; G. Gallagher; S. Kotenko, New Jersey, NJ, USA; S. Chada, Houston, TX, USA; Y. Chandrasekher, Seattle, WA, USA; E. Caudell, Houston, TX, USA; J. Peat, Glasgow, UK; J-C. Renauld, Brussels, Belgium; L. Fouser, Cambridge, MA, USA; and H. Fickenscher, Erlangen, Germany). The human homologs are located on chromosomes one and 12, close to the gene loci encoding IL-10 and IFN-γ, respectively (Fig. 1). Three of these homologs [IL-19, IL-20 and melanoma differentiation-associated antigen 7 (MDA-7)] are produced by stimulated peripheral blood mononuclear cells[2]. None of the related molecules seem to possess the inhibitory activities of IL-10, but their strong antitumor and selective pro-inflammatory activities make them interesting new candidates for research and drug development. Table 1 summarizes the most important properties of these homologs.

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